IX. ABSTRACT

Spinal muscular atrophy (SMA) is a lethal autosomal recessive disease. The gene most highly associated with SMA is the survival motor neuron (SMN) gene. The present invention is a new procedure for the cloning of human SMN, the SMA determining gene, based on the reverse transcription (RT) and the polymerase chain reaction (PCR). This procedure for cloning human SMN gene by means of RT-PCR reactions is cost-effective, not time-consuming, and is suited for any laboratory.

The present invention also includes a new procedure for the construction of expression plasmids, a/ using the pFastBac™ HTb and the pBlueBacHis2 A transfer vectors for the purpose of obtaining human SMN protein in insect cells; and b/ using the pET-28a (+) transfer vector for the purpose of obtaining human SMN protein in bacteria.

The present invention makes it easier to obtain full-length SMN protein which is valuable for biochemical and biological analyses that may elucidate the molecular mechanism of SMA. Knowing the molecular mechanism of SMA will allow the exploration of gene therapy in SMA.

LEGEND OF FIGURES

Figure 1: PCR amplification of the human SMN gene.

M: Marker.

1: PCR product of human SMN gene (~ 0.9 kb).

Figure 2: Cloning of the SMN-cDNA into pCR^{R} II plasmid vector.

The arrow head point is the direction of transcription.

(1): pCR^{R} II/SMN-cDNA.

Figure 3: Construction of pFastBac^{TM} HTb/SMN-cDNA.

The arrow head point is the direction of transcription.

(2): pFastBac^{TM} HTb/SMN-cDNA.

(3): SMN-cDNA in bacmid of DH10Bac^{TM} E. Coli.

Figure 4: Construction of pBlueBacHis2 A/SMN-cDNA.

The arrow head point is the direction of transcription.

(4): pBlueBacHis2 A/SMN-cDNA.

Figure 5: Construction of pET-28a (+)/SMN-cDNA.

The arrow head point is the direction of transcription.

(5): pET-28a (+)/SMN-cDNA.